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AGGLUTININS IN HOG-CHOLERA IMMUNE SERUM FOR BACILLUS SUIPESTIFER *

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The eventual value of hog-cholera immune serum for the treatment of chronic hog cholera makes it seem of advantage to know something concerning the quantity of antibodies against Bacillus suipestifer in the commercial hog-cholera immune serum. So far as agglutinins are concerned, data were obtained by the following tests.

Blood was drawn from the tail or from the jugular vein; it was at once defibrinated and centrifugated. A 10% dilution of the serum in natural salt solution was made and from this further dilutions as follows:

Tube	Salt Solution, c.c.	Serum, c.c.	Bacterial Emulsion, c.c.	Dilution
1	...	0.5 (10%)	0.5	1:20
2	0.5	0.5 (10%)	0.5	1:40
3	0.5	0.5 from Tube 2	0.5	1:80
4	0.5	0.5 from Tube 3	0.5	1:160
5	0.5	0.5 from Tube 4	0.5	1:320
6	0.5	0.5 from Tube 5	0.5	1:640
7	0.5	0.5 from Tube 6	0.5	1:1280
8	0.5	0.5 from Tube 7	0.5	1:2560
9*	0.5	0.5 from Tube 8	0.5	1:5120

* 0.5 c.c. is removed from Tube 9.

The bacterial emulsion was made by washing off agar cultures of B. suipestifer, 24 hours old. The strain was sent to us from the New York Museum of Natural History.

TABLE 1

THE AGGLUTININS IN VIRUS PIGS (AVERAGE WEIGHT 75 LB.)

Pig	Date of Infection	Date of Bleeding	Agglutination Titer
1	April 15	April 26	—
2	Contact infection	April 28	1:40
3	Contact infection	April 28	—
4	April 21	April 28	1:40
5	April 21	April 28	—
6	April 21	April 28	—
7	April 21	April 28	1:20
8	April 21	April 28	—

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* Received for publication April 20, 1916.

TABLE 2

THE AGGLUTININS IN HYPERIMMUNE PIGS (AVERAGE WEIGHT 250 LB.)

Pig	Date of Hyper-immunization	Date of Rehyper-immunization	Date of Bleeding	Agglutination Titer
1	February 4	March 10	April 6	1:160
2	February 6	March 12	April 6	1:1280
3	February 6	March 12	April 6	1:320
4	February 8	March 13	April 6	1:40
5	February 9	March 13	April 6	1:640
6	February 8	March 12	April 6	1:80
7	February 20-25	March 22	April 8	—
8	February 20-25	March 22	April 8	1:160
9	February 25	March 22	April 8	1:160
10	February 25	March 22	April 8	1:320
11	February 25	March 22	April 8	1:1280
12	February 25	March 22	April 8	1:320
13	February 25	March 22	April 8	1:20
14	February 25	March 22	April 8	1:80
15	February 15	March 24	April 9	1:80
16	February 15	March 23	April 9	—
17	February 15	March 23	April 9	1:640
18	February 15	March 23	April 9	1:40
19	February 15	March 23	April 9	—
20	February 20	March 24	April 9	1:20
21	February 20	March 24	April 9	1:1280
22	February 20	March 24	April 9	1:20
23	February 15	March 25	April 12	1:40
24	February 16	March 25	April 12	1:1280
25	February 16	March 25	April 12	—
26	February 16	March 25	April 12	1:20
27	February 16	March 25	April 12	1:20
28	February 20	March 25	April 12	1:80
29	February 23	March 26	April 12	1:20
30	February 23	March 26	April 12	1:160
31	February 8	March 22	April 14	1:1280
32	February 15	March 23	April 14	—
33	February 15	March 23	April 14	1:2560
34	February 15	March 23	April 14	1:40
35	February 17	March 22	April 14	1:80
36	February 17	March 22	April 14	1:160
37	February 17	March 22	April 14	1:80
38	February 17	March 22	April 14	1:40
39	February 17	March 30	April 16	1:160
40	February 17	March 30	April 16	1:320
41	February 17	March 29	April 16	1:1280
42	February 17	March 29	April 16	1:80
43	February 17	March 29	April 16	1:320
44	February 17	March 29	April 16	1:320
45	February 28	March 30	Opril 16	1:5120
46	February 28	March 30	April 16	1:1280
47	March 1	March 31	April 17	1:640
48	March 1	March 31	April 17	1:1280
49	March 1	March 31	April 17	1:160
50	March 1	March 31	April 17	1:5120
51	March 1	March 31	April 17	1:640
52	March 1	March 31	April 17	1:6000
53	March 1	March 31	April 17	1:640
54	March 3	April 3	April 17	1:160
55	February 17	April 1	April 19	1:640
56	February 17	April 1	April 19	1:6000
57	February 17	April 1	April 19	1:10000
58	March 1	April 1	April 19	1:2560
59	March 3	April 3	April 19	1:80
60	March 2	April 2	April 19	1:160
61	March 2	April 2	April 19	1:320
62	March 2	April 2	April 19	1:160
63	February 10	April 10	April 20	1:80
64	March 6	April 10	April 20	1:320
65	March 3	April 3	April 20	1:320
66	March 3	April 3	April 20	1:640
67	March 3	April 3	April 20	1:20
68	March 3	April 3	April 20	1:320
69	March 3	April 3	April 20	1:160

TABLE 2—*Continued*

THE AGGLUTININS IN HYPERIMMUNE PIGS (AVERAGE WEIGHT 250 LB.)

Pig	Date of Hyperimmunization	Date of Rehyperimmunization	Date of Bleeding	Agglutination Titer
70	March 3	April 3	April 20	1:160
71	February 17	March 30	April 23	1:320
72	February 17	March 29	April 23	1:1280
73	March 1	March 31	April 23	1:640
74	March 1	March 31	April 23	1:1280
75	March 1	March 31	April 23	1:80
76	March 1	March 31	April 23	1:5120
77	March 1	March 31	April 23	1:320
78	March 1	March 31	April 23	1:2560
79	February 17	April 15	April 24	1:2560
80	February 17	April 15	April 24	—
81	February 19	April 15	April 24	1:20
82	March 6	April 14	April 24	1:320
83	March 6	April 14	April 24	1:640
84	March 6	April 15	April 24	1:2560
85	March 6	April 15	April 24	1:20
86	March 6	April 15	April 24	1:2560
87	February 17	April 16	April 26	1:320
88	February 17	April 16	April 26	1:40
89	February 17	April 16	April 26	—
90	February 17	April 17	April 26	1:80
91	February 19	April 17	April 26	1:2560
92	February 19	April 17	April 26	1:160
93	February 19	April 16	April 26	1:20
94	February 17	April 7	April 27	1:5120
95	March 2	April 2	April 27	1:160
96	February 17	April 1	April 27	1:640
97	February 17	April 2	April 27	1:640
98	February 17	April 1	April 27	1:1280
99	February 17	April 1	April 27	1:80
100	February 17	April 1	April 27	1:320

Of 8 normal pigs, 5 showed no agglutination of B. suipestifer, while 2 gave agglutination in 1: 10, and 1 in 1: 40.

As Tables 1 and 2 indicate, the high titers were present only in the sera of hyperimmune pigs. The agglutinins were produced not as a result of acute cholera, but as a result of the presence of B. suipestifer in the large amount of virus blood injected (5 c.c. to each pound of the pig). In the average the titer was 1: 1000, or exactly 1: 999.5. Twenty-one pigs had a titer under 80, and in these cases I assume that no considerable quantity of B. suipestifer had been injected. Often the blood of one virus pig was used for the hyperimmunization of one immune pig. Mixtures of different blood specimens were however usual. In our case it could be rightly supposed that in 21% of the hyperimmunizations the blood was practically free from B. suipestifer. This percentage, representing the absence of B. suipestifer in 100 cases of hog cholera, is of course too low and this circumstance is most likely due to mixture of the viruses and to the persistence of agglutinins from the first hyperimmunization. However, I have reason to believe that here in Ames the percentage of cases of hog cholera mixed with secondary infections of the paratyphosus-B. type is above 44.6%

(Uhlenhuth's number). Eberson[1] found under the same conditions 28 of 55 virus pigs infected with B. paratyphosus B.

To demonstrate any relation that might exist between the strength of the hyperimmune serum and the agglutinating titer for B. suipestifer, the following experiment was made:

From 8 immune pigs ready to be hyperimmunized, blood was taken and the agglutinating titer for B. suipestifer found. The titers were as follows:

Pig	1:10	1:20	1:40	1:80	1:160	1:320	1:640	Titer
1	+++	+++	++	+	—	—	—	1:80
2	+++	+++	+	—	—	—	—	1:40
3	++	+	—	—	—	—	—	1:20
4	++	+	—	—	—	—	—	1:20
5	+++	+++	+++	+++	++	+	—	1:320
6	++	++	+	—	—	—	—	1:40
7	+++	++	+	—	—	—	—	1:40
8	++	+	—	—	—	—	—	1:20

The pigs, which all weighed near 200 pounds, were at the same time hyperimmunized with 5 c.c. of virus blood to 1 pound of body weight. The virus blood was taken from several pigs and carefully mixed, so that each pig would get the same amount of virus and bacteria. After 10 days blood was drawn again from the hyperimmunized pigs and the agglutinating power tested. Three pigs were accidentally lost. The titers of the 5 left were as follows:

Pig	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560	1:5120	1:6000	1:8000	1:10000
1	+++	++	+	—	—	—	—	—	—			
4	++	+	—	—	—	—	—	—	—			
5	+++	+++	+++	+++	+++	+++	+++	+++	++	++	+	—
7	+++	+++	+++	+++	++	—	—	—	—			
8	+++	++	—	—	—	—	—	—	—			

Then the strength of these sera as hog-cholera immune sera was tested. Eight young pigs of about equal weight were selected for each serum and pairs of them injected severally with different amounts of serum and 2 c.c. virus. The virus was previously tested out to determine its virulence. The results are given in Table 3.

All the test pigs showed febrile reactions. The cause of the deaths was ascertained by postmortem examinations.

Serum 7 and Serum 8 were the strongest so far as protective power against hog-cholera virus is concerned, a fact which is not in accordance with the result of the agglutination tests.

The experiment was not carried out further, because conclusions were reached from other experiments (report of which will be published in the near future) indicating the improbability of a connection,

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[1] Jour. Infect. Dis., 1915, 17, p. 331.

TABLE 3

PROTECTIVE POWER OF HYPERIMMUNE SERA AGAINST HOG CHOLERA

Test Pigs	Amount of Serum in c.c.	Result
Serum 1		
1	20	—
2	20	—
3	15	—
4	15	Died after 16 days. H. C.
5	10	—
6	10	—
7	5	Died after 18 days. H. C.
8	5	Died after 10 days. H. C.
Serum 4		
9	20	—
10	20	—
11	15	—
12	15	—
13	10	—
14	10	—
15	5	Died after 13 days. H. C.
16	5	Died after 10 days. H. C.
Serum 5		
17	20	—
18	20	—
19	15	—
20	15	—
21	10	—
22	10	—
23	5	Died after 15 days. H. C.
24	5	Died after 14 days. H. C.
Serum 7		
25	20	—
26	20	—
27	15	—
28	15	—
29	10	—
30	10	—
31	5	—
32	5	—
Serum 8		
33	20	—
34	20	—
35	15	—
36	15	—
37	10	—
38	10	—
39	5	—
40	5	—

and because the negative outcome had a much higher determining value than an eventual positive one. Pfeiffer and Kolle and Dieudonné[2] have shown that there is no connection between immunity and agglutinins;

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[2] Cited by Paltauf, Kolle und Wassermann's Handb. d. pathogen. Mikro-organismen, 1913, 2, p. 483.

the same authors and many others have demonstrated that the amount of bactericidal immune bodies is not analogous to the amount of agglutinins in an immune serum against the same antigen.

SUMMARY

The paper presents tabulations of the agglutinins for B. suipestifer in 100 hog-cholera immune sera, in 8 sera of normal pigs, and in 8 sera of virus pigs, also the report of an experiment to ascertain the connection between the agglutinin titer for B. suipestifer and the amount of virus antibodies in a hyperimmune serum.